PESTICIDE RESIDUES IDENTIFIED IN GRAPE VARIETIES

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Abstract

As the human population continues to grow there is an ever-increasing demand for food production, which means that more and more pesticides will be required to meet these needs. This trend is the same in the winemaking industry with pesticides being used in higher quantities year after year. As pesticides can produce harmful effects on human beings and the environment it is crucial to accurately understand how pesticides travel through the winemaking process. This research sought to monitor the pesticides from two grape varieties. The following six pesticides were analysed: oxathiapiprolin, myclobutanil, iprovalicarb, tebuconazole, chlorantraniliprole, and acetamiprid. Samples were extracted using the QuEChERS (quick, easy, cheap, effective, rugged, and safe) method and analysed for the residues of pesticides by liquid chromatography-tandem mass spectrometry. Results indicated that pesticides content in the grape samples ranged between 0.89 ng/g and 18.92 ng/g for Feteasca neagra grape variety. Similar for Cabernet Sauvignon grape values ranged between 0.66 ng/g and 8.24 ng/g. Overall, the recorded levels of pesticides were significantly below the EU maximum residue levels (MRL's).

Key words: pesticide residues, grapes, QuEChERS, LC-MS/MS.

Grapes are an important source of polyphenols and other health beneficial compounds, however, grapevine is a very susceptible crop to a large number of pathogens (Dumitriu D. *et al*, 2015).

The fruits and aerial parts of the grapevine can become infected by diseases such as downy mildew (*Plasmopara viticola*), Botrytis rot (*Botrytis cinerea*), black rot (*Guignardia bidwellii*), Eutypa dieback (*Eutypa lata*), Phomopsis cane/leaf spot (*Phomopsis viticola*), powdery mildew (*Unicola necator*) or sour rot (*Aspergillus niger, Alternaria tenius, Botrytis cinerea, Cladosporium herbarum, Rhizopus arrihizus,* and *Penicillium* spp.) (Fermaud M. *et al*, 2016).

From an economical perspective, fungal diseases cause significant yield and grape quality losses in grapevine or can increase the production cost through the use of antifungal treatments.

According to the European Commission (EC, 2021) pesticides can be defined as something that prevents, destroys, or controls a harmful organism ('pest') or disease, or protects plants or plant products during production, storage and transport.

There are many different types and categories of pesticides which are each designed to target a specific 'pest'. Herbicides are the most common variant and are widely used in agriculture and wildland ecosystems in order to ensure that weed density is reduced, and desirable species are encouraged to proliferate (Holt J., 2013). Insecticides are also widely used in the agriculture sector and are designed to mitigate, repel or kill one or more species of insect (National Pesticide Information Center - NPIC, 2019). Fungicides are another common type of pesticide which target and eliminate various types of mould, mildew and other fungi (Canadian Centre for Occupational Health and Safety - CCOHS, 2017). Other common types of pesticides include acaricides, nematicides, molluscicides, rodenticides, growth regulators, repellents and rodenticides (EC, 2021) which are used across a variety of industries for numerous purposes. Pesticides have been used since as far back as 4500 years ago, when the Sumerians used sulphur dust to control insects and mice, and 3000 years ago when the Chinese used mercury and arsenic to control lice (International Union of Pure and Applied Chemistry - IUPAC, 2010). Since then, major scientific breakthroughs have identified more effective chemical compounds, and more reliable investigation tools, that resulted in the discontinuation

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commercialisation of several of these chemicals (Stockholm Convention, 2019).

While pesticides have a number of extremely useful attributes, they are also harmful to individuals and the environment, and their presence in food is especially hazardous (Fenik J. *et al*, 2011). Many pesticides have been found to have severe effects with regards to endocrine disorders, reproductive problems, cancers, diabetes, obesity and cardiovascular diseases (Thakur M. *et al*, 2020). There are a number of ways that pesticides can be categorized in terms of risk, according to different governing bodies such as the Environmental Protection Agency of the United States of America (EPA) and World Health Organization (WHO), ranging from 'unlikely to present acute hazard' to 'extremely hazardous' depending on their properties. They can be classified together in a number of ways; by target pest group, by mode of action, or by chemical family. In order to safeguard human health, while still facilitating world trade, the WHO and the Food and Agriculture Organization (FAO) have set up a joint Codex Alimentarius Commission to elaborate the necessary food standards, as well as suggesting the Maximum Residue Levels (MRLs) of pesticides legally allowed in or on food products or animal feed.

The production of grapes and their by-products require a large amount of pesticide use worldwide. In fact, data illustrating the unprocessed food products most frequently containing pesticide residue shows that approximately 65.7% of table and wine grapes contain pesticide residue.

The aim of this work is to monitor the content of pesticides from grape samples. The pesticides content in the sample is extracted in acetonitrile and purified with the solid-phase extraction cartridge using the QuEChERS method and detected by liquid chromatography-tandem mass spectrometry.

MATERIAL AND METHOD

Fetească neagră and Cabernet Sauvignon (*Vitis vinifera*) grapes were harvested in 2020 from the experimental vineyard located in Ferma Adamachi, Iasi, belonging to Ion Ionescu de la Brad University of Life Sciences Iasi (IULS). The experimental protocol included eight treatments in the vineyard from April to August 2020. The vineyard had 12 ha in area and was treated with the following phytosanitary products: Zorvec Zelavin (oxathiapiprolin), Systhane (myclobutanil), Melody Compact (8.5% iprovalicarb + 40.6% copper oxychloride), Folicur (tebuconazole), Coragen (chlorantraniliprole), and Gazelle (acetamiprid) using the doses recommended by the manufacturer and preserving thus the pre-harvest time in vines. Grapes were harvested at the end of September in good sanitary conditions.

The standards of pesticides were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The Quick QuEChERS-Medium Cartridge was from United Chemical Technologies (Bristol, PA, USA).

The extraction and identification of pesticides, both described previously in detail by Dumitriu Gabur G.D. *et al*, 2022.

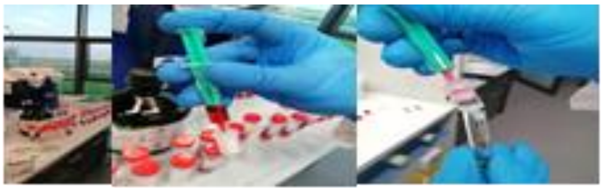

Figure 1 **Preparation of grape samples**

The obtained extract from QuEChERS was placed into an autosampler vial and injected into the LC-MS/MS system. The UHPLC Flexar chromatographic system (Perkin Elmer, Waltham, MA, USA) with a Kinetex biphenyl column was used for the identification of pesticides. The detection was made by an AB SCIEX Triple Quad 5500 mass spectrometer (Framingham, MA, USA).

RESULTS AND DISCUSSIONS

In vineyards throughout the cropping season, insecticides, herbicides and fungicides are often applied for the management of pathogens and maintenance of pests. In 2018, approximately 400,000 tonnes of pesticides were sold in Europe, predominantly to the agricultural sector (PAN Europe, 2020).

Different phytosanitary treatments were carried out during the growing season of *Vitis vinifera* var. Fetească neagră and Cabernet Sauvignon grapes in 2020. For Cabernet Sauvignon grapes the following oenological parameters were obtained: total and volatile acidities of 5.44 g/L and 0.25 g/L of tartaric and acetic acid, respectively; pH 3.58; ethanol content 12.16 % v/v; total dry extract 26.1 g/L; reducing sugars 2.13 g/L. For Fetească neagră wines the following oenological parameters were obtained: total acidity and volatile acidity of 4.03 g/L and 0.35 g/L of tartaric and acetic acid, respectively; pH 3.63; ethanol content 13.52 % v/v; total dry extract 24.8 g/L; reducing sugars 2.16 g/L.

As we can see from Figure 2, quantities of pesticides within the Fetească neagră grapes are expressed here in ng/g, while the MRLs are expressed in mg/kg, where 1 ng/g is equal to 0.001 mg/kg.

With respect to the remaining pesticides, Acetamiprid residues were 4.291 ng/g (0.004291 mg/kg) which is below the EU MRL of 0.5 mg/kg, Chlorantraniliprole residues were 18.921 ng/g (0.018921 mg/kg) which is below the EU MRL of 1 mg/kg, Iprovalicarb residues were 0.891 ng/g (0.000891 mg/kg) which is below the EU MRL of 2 mg/kg, Myclobutanil residues were 2.324 ng/g (0.002324 mg/kg) which is below the EU MRL of 1 mg/kg, Tebuconazole residues were 4.266 ng/g (0.004266 mg/kg) which is below the EU MRL of 0.5 mg/kg, and Oxathiapiprolin residues were 6.476 ng/g (0.006476 mg/kg) which is below the EU MRL 0.7 mg/kg. Fluazifop-butyl were not found in our study (*Figure 2*).

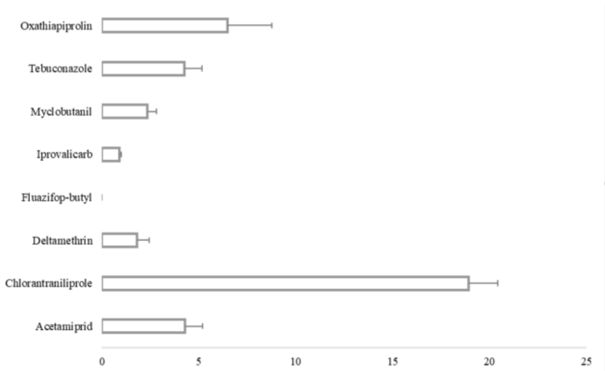

Figure 2 **Pesticide residues quantified in grapes of Feteasca neagra**

As with the Feteascǎ neagrǎ, the levels of pesticides recorded in the Cabernet Sauvignon grapes were all below EU MRLs. Once again Deltamethrin and Fluazifop-butyl were not found in our grape.

With respect to the remaining pesticides, Acetamiprid residues were 1.185 ng/g (0.001185 mg/kg) which is below the EU MRL of 0.5 mg/kg, Chlorantraniliprole residues were 8.247 ng/g (0.008247 mg/kg) which is below the EU MRL of 1 mg/kg, Iprovalicarb residues were 0.659 ng/g (0.000659 mg/kg) which is below the EU MRL of 2 mg/kg, Myclobutanil residues were 1.166 ng/g (0.001166 mg/kg) which is below the EU MRL of 1 mg/kg, Tebuconazole residues were 2.165 ng/g (0.002165 mg/kg) which is below the EU MRL of 0.5 mg/kg, and Oxathiapiprolin residues were 3.599 ng/g (0.003599 mg/kg) which is below the EU MRL 0.7 mg/kg (*Figure 3*).

A number of studies have been carried out concerning the residues of pesticides in grapes, in order to monitor the content of pesticides and how can we prevent their penetration into the wine. Cabras P. *et al*, 2000 assessed 47 samples of grapes for winemaking and found that after the fermentation process, pesticides like mepanipyrim, fluazinam, and chlorpyrifos were not detected in wines, but most of the pesticides pass from grape to must and wine.

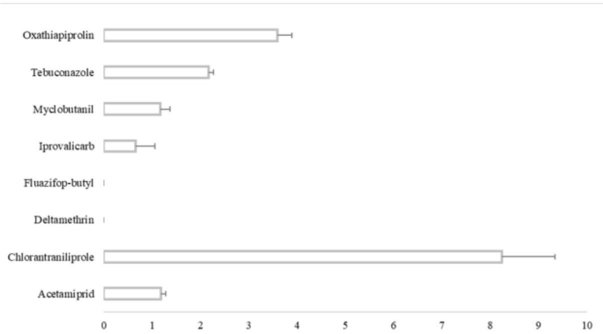

Figure 3 **Pesticide residues quantified in grapes of Cabernet Sauvignon**

Cesnik H.B. *et al*, 2008 also monitored 47 grape species for pesticide residues in vineyards included in integrated crop management and found that chlorothalonil dithio-carbamates, cyprodinil, folpet, chlorpyriphos, and pyrimethanil were the most detected in these samples.

Urkude R. *et al*, 2019 examined the persistence of pesticides in different areas of India and reported the presence of tebuconazole and kresoxim methyl in grapes, however both were below the quantifiable limit.

Determining residue levels in food and beverages is a complicated and time-consuming process. Winemaking carried out without the skins results only in pesticides which have passed through via the must, whereas winemaking carried out with the skins can contain all the pesticide residue of the grape. Generally, the level of pesticides found in wine is lower than the levels found on the grapes and in the must (Urkude et al., 2019). According to Urkude et al. (2019), pesticide residues stay on or in the final produce when the pesticide dose, preharvest period, or appropriate agricultural practices are not followed, resulting in deterioration of wine quality, environmental effects, and consumer health concerns. While most pesticides are degraded during the winemaking process, some residual levels remain, therefore it is essential to determine pesticide residues at harvest time to ensure safe consumption.

CONCLUSIONS

Compared with the maximum residue limits (MRLs), none of the detected pesticide residues in grape samples exceeded the MRLs.

Pesticide use is predicted to continue to increase globally in the coming year, however, as new information and research comes to light about the serious detrimental effects of pesticides, the European Commission has outlined an in-depth plan to reduce pesticides by 50% in the next decade. The plan sets out guidelines to tackle the key drivers of biodiversity loss such as unsustainable use of land and sea, overexploitation of natural resources, pollution, and invasive alien species, as well as to enable the transition to a sustainable EU food system that safeguards food security and ensures access to healthy diets.

It is also crucial that legislation remains progressive and continues to adapt to further reduce the harmful effects of pesticides to both human health and to the environment, particularly as pesticide use continues to increase globally.

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